

CLAIMS

WHAT IS CLAIMED IS:

1. A multiplex structure comprising:
a first strand containing a first sequence of
nucleobases.

5 a second strand containing a second sequence of
nucleobases, wherein said second strand is
associated with said first strand by Watson-Crick
bonding;

10 a third strand containing a third sequence of
nucleobases; and

a fourth strand containing a fourth sequence of
nucleobases, wherein said fourth strand is
associated with said second strand and said third
strand by Watson-Crick bonding.

15 2. The multiplex structure of claim 1, wherein said
multiplex structure is an isolated, purified, artificial or
synthetic quadruplex.

20 3. The multiplex structure of claim 1, wherein each
said strand independently comprises a nucleic acid or a
nucleic acid analogue.

4. The multiplex structure of claim 3, wherein each
said strand independently comprises DNA or RNA.

25 5. The multiplex structure of claim 3, wherein each
said strand independently comprises a nucleic acid analogue
containing an uncharged or partially charged backbone.

30 6. The multiplex structure of claim 1, wherein one
of said second strand or said fourth strand comprises DNA
and the other of said second strand or said fourth strand
comprises RNA, mRNA, hnRNA, rRNA, tRNA or cDNA.

7. The multiplex structure of claim 1, wherein said
second strand and said fourth strand are anti-parallel to
each other.

8. The multiplex structure of claim 7, wherein a major groove of said first strand and said second strand is placed in a major groove of said third strand and said fourth strand.

5 9. The multiplex structure of claim 1, wherein said second strand and said fourth strand are parallel to each other.

10 10. The multiplex structure of claim 9, wherein a major groove of said first strand and said second strand is placed in a minor groove of said third strand and said fourth strand.

W/M by? 11. The multiplex structure of claim 1, wherein each nucleobase binds to no more than two other nucleobases.

15 12. The multiplex structure of claim 1, wherein no strand is contiguous with another strand.

13. The multiplex structure of claim 1, wherein said multiplex structure is substantially free of Hoogsteen bonding.

20 14. The multiplex structure of claim 1, wherein said multiplex structure is substantially free of G-G quartets.

15. The multiplex structure of claim 1, wherein said first strand and said second strand are 5 to 50 base pairs long.

25 16. The multiplex structure of claim 1, wherein said third strand and said fourth strand are genomic DNA.

17. The multiplex structure of claim 1, wherein said third strand and said fourth strand include a haplotype in genomic DNA.

30 18. The multiplex structure of claim 1, wherein said third strand and said fourth strand are PCR amplified products.

19. The multiplex structure of claim 1, wherein said multiplex structure is free of solid support.

20. The multiplex structure of claim 1, wherein said multiplex structure is bound to a solid support.

21. The multiplex structure of claim 1, wherein said solid support is not electrically conductive.

5 22. The multiplex structure of claim 1, wherein said solid support is electrically conductive.

10 23. The multiplex structure of claim 1, further comprising a therapeutic, prophylactic or diagnostic agent bound to at least one of said first strand, said second strand, said third strand and said fourth strand.

15 24. The multiplex structure of claim 1, wherein said first strand and said second strand are each 5 to 30 bases long and said third strand and said fourth strand are each 8 to 3.3×10^9 base pairs long.

25. The multiplex structure of claim 1, wherein said fourth sequence contains 25% to 75% purine bases and 75% to 25% pyrimidine bases in any order.

20 26. A method for providing the multiplex structure of claim 1, said method comprising:

providing a hybridization medium comprising said first strand, said second strand, said third strand, said fourth strand, water, a buffer and at least one promoter; and

25 incubating said hybridization medium for an incubation time effective to hybridize said second strand to said fourth strand to provide said multiplex structure.

30 27. The method of claim 26, wherein said hybridization medium is buffered to a pH of about 5 to about 9.

28. The method of claim 26, wherein said at least one promoter is an intercalating agent.

29. The method of claim 28, wherein said at least one promoter is an intercalating fluorophore, and a fluorescent intensity of a test medium containing said multiplex structure is directly correlated with a binding affinity of
5 said second strand for said fourth strand.

30. The method of claim 29, wherein said intercalating fluorophore is a member selected from the group consisting of YOYO-1, TOTO-1, ethidium bromide, ethidium homodimer-1, ethidium homodimer-2 and acridine.

10 31. The method of claim 26, wherein said at least one promoter is tethered to at least one of said first strand, said second strand, said third strand and said fourth strand.

15 32. The method of claim 26, wherein said at least one promoter is a monovalent cation.

33. The method of claim 26, wherein said at least one promoter is a cation having a valency greater than one.

20 34. The method of claim 33, wherein said cation is at least one member selected from the group consisting of alkali metal cations, alkaline earth metal cations, transition metal cations, $\text{Co}(\text{NH}_3)_6^{+3}$, trivalent spermidine and tetravalent spermine.

35. The method of claim 33, wherein said cation is K^+ or Na^+ provided at a concentration of 50mM to 125mM.

25 36. The method of claim 26, wherein said third strand and said fourth strand are provided in said hybridization medium before said first strand and said second strand, and wherein said first strand and said second strand are provided in dehydrated form prior to rehydration by contact
30 with said hybridization medium.

37. The method of claim 26, wherein said incubation time is not more than about two hours.

38. The method of claim 26, wherein said incubating is conducted at room temperature.

5 39. The method of claim 26, wherein hybridization of said second strand to said fourth strand is detected as a change in a fluorescent, chemiluminescent, electrochemiluminescent or electrical signal.

40. The method of claim 39, wherein an intensity of said signal is correlated with a binding affinity between said second strand and said fourth strand.

10 41. The method of claim 40, wherein at least one of said first strand and said second strand is covalently labeled with a non-intercalating fluorophore and said intensity is inversely correlated with said binding affinity.

15 42. The method of claim 41, wherein said non-intercalating fluorophore is a member selected from the group consisting of biotin, rhodamine and fluorescein.

20 43. The method of claim 41, wherein said method is a homogeneous assay conducted without providing a signal quenching agent on said target sequence or on said probe.

44. The method of claim 26, wherein hybridization of said second strand to said fourth strand inactivates an activity associated with at least one of said third strand and said fourth strand.

25 45. The method of claim 26, wherein at least one of said first strand and said second strand further comprises a pharmaceutical agent, and wherein hybridization of said second strand to said fourth strand places said pharmaceutical agent an effective distance from a target on said third strand, said fourth strand or on another molecule associated with at least one of said third strand and said fourth strand.

46. The method of claim 45, wherein said pharmaceutical agent is a member selected from the group consisting of nucleic acids designed to bind promoter sequences of clinically relevant genes, nucleic acids designed to bind clinically relevant genes, or nucleic acids designed to bind origin of replication sites of pathogens.

5 47. The method of claim 26, wherein a ratio of said first strand and said second strand to said third strand and said fourth strand is about 10:1.

10 48. The method of claim 26, wherein concentrations of each of said first strand, said second strand, said third strand and said fourth strand are not more than 5×10^{-10} M.

15 49. The method of claim 26, wherein said at least one promoter is a minor groove nucleic acid binding molecule, which binds in a non-intercalating manner and binds with an association constant of at least 10^3 M⁻¹.

20 50. The multiplex structure of claim 1, wherein said first strand is associated with said third strand by Watson-Crick bonding.

51. An electrical circuit comprising the multiplex structure of claim 1.

52. A method for assaying binding, said method comprising:

25 providing a target nucleic acid or nucleic acid analogue having a target sequence, wherein said target sequence contains at least one purine base and at least one pyrimidine base;

providing a double-stranded probe comprising a nucleic acid sequence or a nucleic acid analog sequence;

30 providing a hybridization promoter;

adding said probe, said target and said hybridization promoter to a medium to provide a test sample

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containing a Watson-Crick triplex or quadruplex comprising said probe bound to said target sequence;

irradiating said test sample with exciting radiation
5 to cause test sample to emit fluorescent radiation;

detecting an intensity of said fluorescent radiation,
wherein said intensity is correlated with a binding affinity between said probe and said target sequence; and

10 determining from said intensity an extent of matching between said probe and said target sequence.

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